THE CONDOR
A JOURNAL OF AVIAN BIOLOGY
Volume 90 Number 3 August 1988

The Condor 90:529–537

FAT FROM BLACK-CAPPED CHICKADEES: AVIAN BROWN ADIPOSE TISSUE?[1]

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Abstract. We investigated the claim that the furcular fat deposits of Black-capped Chickadees (*Parus atricapillus*) contain brown adipose tissue (BAT) in winter. Electron micrographs show that mitochondrial densities in these deposits are substantially lower than those in mammalian BAT. Protein-specific activities of the catabolic enzymes citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (HOAD) in furcular fat are only 25% and 39%, respectively, of the corresponding values for pectoralis muscle of chickadees in January. The differences between chickadee tissues are more marked when activities are compared in birds collected later in winter (February to March). Catabolic capacities of furcular fat in either winter sample are an even smaller fraction of those documented in BAT from winter-acclimatized deer mice (*Peromyscus leucopus*). Near-maximal stores of furcular fat in chickadees differ from depleted ones in having lower mitochondrial densities and fewer lipid droplets, as well as lower protein concentrations and lower protein-specific CS and HOAD activities. The histological characteristics and low catabolic capacities of furcular fat in chickadees suggest that this tissue is an unlikely site of intense thermogenesis. We question the claim (Oliphant 1983) that it is a functional equivalent of mammalian BAT.

Key words: Parus atricapillus; *Black-capped Chickadee; brown adipose tissue; nonshivering thermogenesis; electron microscopy; catabolic capacity.*

INTRODUCTION

Small birds living in arctic or continental temperate climates are subject to high rates of heat loss during winter. Consequently, maintenance of thermal balance in this season involves sustaining high rates of thermogenesis. Shivering is regarded as the principal mode of thermogenesis in birds (Hart 1962, West 1965, Rautenberg 1969), with regulatory nonshivering thermogenesis (NST) either absent or, if present, less important. The lack of a calorigenic response to norepinephrine (Hart 1962, Rautenberg 1969, Hissa and Palokangas 1970, Chaffee and Roberts 1971, Koban and Feist 1982) as well as the failure to detect brown adipose tissue (BAT) in six species of birds (Johnston 1971) suggest that any NST must differ in pattern from that evident in mammals. These data, however, are inconclusive. For instance, the animals studied by Johnston (1971) were collected in late March to late September and in the southern United States, a time of year and a region in which birds would not face severe cold. Furthermore, another hormonal agent (e.g., glucagon) may exert a calorigenic role in place of norepinephrine in some birds (Barré et al. 1987).

Using histological and electron microscopic techniques, Oliphant (1983) observed tissue in one Ruffed Grouse (*Bonasa umbellus*) and two Black-capped Chickadees (*Parus atricapillus*) that appeared similar to mammalian BAT. Ultrastructural features resembling those of mammalian BAT included high capillary densities and the presence of numerous microvilli on the capillary walls. At the cellular level, the avian adipocytes were polygonal and multilocular, had

[1] Received 28 September 1987. Final acceptance 11 April 1988.

centrally located nuclei and, in Oliphant's opinion, relatively high mitochondrial densities. These features persuaded him that avian adipose tissue possesses high capacities for the endogenous oxidation of fat, making it a potential site for intense thermogenesis. The implications of these findings for understanding mechanisms of avian cold defense prompted us to investigate further Oliphant's claim that birds possess BAT.

We report here results of our examination of the furcular (claviculocoracoid) fat deposit of Black-capped Chickadees. We have sought to determine whether or not: (1) avian adipocytes share the ultrastructural characteristics typical of mammalian BAT; and (2) avian adipose tissue shares the high catabolic enzyme capacities evident in mammalian BAT (Wickler 1979). To evaluate the first question, electron micrographs (EMs) of furcular fat were prepared from chickadees collected during the colder months in Michigan. For the second question, tissues from additional winter-acclimatized chickadees were assayed for citrate synthase and β-hydroxyacyl-CoA-dehydrogenase activities.

Black-capped Chickadees are well-suited for this study. As small, year-round residents of northern temperate forests, these birds are especially subject to severe thermal stresses during the winter. Like other members of the family Paridae (Steen 1958, Haftorn 1972, Reinertsen 1983, Reinertsen and Haftorn 1986), *P. atricapillus* can husband its energy reserves on cold winter nights by entering nocturnal hypothermia down to 12°C below normothermic body temperatures (Chaplin 1974, 1976). Varying the intensity of shivering thermogenesis is one mechanism by which parids control the entrance into, maintenance of, and arousal from nocturnal hypothermia (Haftorn 1972, Chaplin 1976). Metabolic rates increase significantly during arousal, especially in smaller parids (Reinertsen 1983). If BAT is present in the furcular region of chickadees, NST in this tissue could contribute significantly to reestablishing normothermic body temperature in arousing birds.

MATERIALS AND METHODS

ANIMALS

Thirty-two chickadees (9.6 to 12.8 g) were collected between 09:45 and 15:46 EST in traps or mist nets in or near Ann Arbor, Washtenaw County, Michigan. Five animals collected in 1984 to 1985 between 1 November and 3 April were used for the ultrastructural analysis of furcular fat. Twenty-four birds were collected between 28 February and 10 March 1985 ($n = 7$; mean body mass $\pm$ SE = 11.25 $\pm$ 0.245 g), 20 February and 17 March 1986 ($n = 8$; 11.32 $\pm$ 0.224 g), and 20 and 28 January 1987 ($n = 9$; 11.12 $\pm$ 0.309 g), and used in the analysis of enzyme activity in pectoralis muscle and furcular fat. The remaining three birds were collected on 24 February 1988 (11.25, 11.74, and 12.43 g) and, along with the 12 birds collected in January and February 1985 to 1987 from above, were used to evaluate the relationship between capture time and furcular fat mass. Minimum air temperatures on the dates of capture (NOAA National Climatical Data Center, 1984–1987) were similar to long-term average minima (over the 99 years prior to 1980) for the corresponding dates in Ann Arbor (see Dawson and Marsh 1986). Minimum daily temperatures ranged from -16.0 to -7.2°C (January 1987 sample), -7.2 to 2.2°C (February to March 1984, 1985, and 1986), and were -8.3, -1.6, and 8.9°C on 24 February 1988, 4 April 1984, and 1 November 1984, respectively. All birds were transported back to the laboratory alive on the day of capture and were used immediately.

ELECTRON MICROSCOPY

Birds were killed by decapitation, and their furcular adipose tissue removed and immediately placed in several drops of fixative (5% glutaraldehyde in 0.2 M s-collidine buffer). Tissue was then minced into 1-mm^3 blocks (8–10 per furcular fat pad) and placed in vials of fresh fixative at room temperature for 2 hr. After fixation, the tissue blocks were washed in several changes of collidine buffer, postfixed with 1% osmium tetroxide in collidine buffer, dehydrated, and embedded in Epon/Araldite. Blocks were semithin-sectioned (1 μm thick) and stained with Toluidine Blue. Blocks obtained from an individual were similar ultrastructurally, so representative semithin sections were subsequently thin-sectioned and stained with uranyl acetate and lead citrate.

ENZYME ACTIVITIES AND PROTEIN CONCENTRATION

Birds were sacrificed by thoracic compression and all of the furcular fat tissue was immediately

removed. Pectoralis muscle was also dissected from birds collected in 1986 and 1987. Tissues were weighed to the nearest 0.1 mg and either used immediately (most 1985 birds) or frozen at −70°C (February 1985, and all 1986–1987 birds). Independent measurements of five muscle and fat tissues established that the activities of the enzymes studied were not affected by storage for 4 months at −70°C. Tissue samples were subsequently thawed, minced, homogenized in 10 or 20 volumes of buffer (100 mM potassium phosphate, 2 mM EDTA at pH 7.3), and sonicated for three 15-sec intervals separated by 45-sec pauses. Samples were maintained on ice until assayed to prevent thermal denaturation during homogenization and sonication.

Enzyme activities were measured spectrophotometrically at 25°C and at 35°C (1987 only) in a final volume of 1 ml. Activity of citrate synthase (CS; E.C. 4.1.3.7) served as an indicator of the catabolic potential of the tricarboxylic acid (TCA) cycle. Activity was measured at 412 nm according to a modification of the method of Srere (1969). Assays were performed in a medium containing 100 mM Tris-HCl, 10 mM potassium phosphate, 0.2 mM s-acetyl CoA, 0.1 mM 5,5′-dithiobis (2-nitrobenzoic acid), 2.5 mM EDTA, 0.5 mM cis-oxaloacetate, and 0.1 ml homogenate, pH 7.3. After equilibration, the reaction was begun by adding the oxaloacetate.

Activity of β-hydroxyacyl-CoA dehydrogenase (HOAD; E.C. 1.1.1.35), used as an index of the capacity for beta oxidation, was measured at 340 nm according to a modification of the method of Bass et al. (1969). Assays were carried out in a reaction medium containing 100 mM triethanolamine-HCl, 5 mM EDTA, 0.45 mM NADH, 0.1 mM s-acetoacetyl CoA, and 0.1 ml homogenate, pH 7.0. After equilibration, the reaction was begun by adding the s-acetoacetyl CoA.

Protein concentration of the furcular fat and pectoralis samples was determined by the Folin phenol reagent method of Lowry et al. (1951). Enzyme activities are expressed as μmoles of substrate converted $(\text{min} \cdot \text{mg protein})^{-1}$.

STATISTICAL ANALYSIS

Differences among tissues and years were evaluated on the Michigan Interactive Data Analysis System (MIDAS, Fox and Guire 1976) using parametric tests. To test for tissue differences, comparisons were made between pectoralis muscle and furcular fat of chickadees and between chickadee furcular fat and interscapular BAT from *Peromyscus leucopus* (Wickler 1979). Whole body masses, furcular fat masses, and enzyme activities of 1985 and 1986 birds did not differ statistically ($P > 0.05$). Consequently, data for these birds were combined in a single February–March sample. The remaining nine birds collected in 1987 constituted the January sample. All 15 birds collected in January and February in the years 1985 to 1988 were used in the regression analysis of capture time and furcular fat mass.

RESULTS

BODY AND TISSUE MASSES

Whole body masses of chickadees collected in late February–March (11.29 ± 0.16 g, $\bar{x} \pm$ SE; $n = 15$) were not significantly different from those of birds collected in January (11.12 ± 0.31 g; $n = 9$; $P > 0.59$; t-test). Furthermore, the amount of furcular fat in the February–March sample (0.0504 ± 0.0054 g; $n = 15$) did not differ from that in the January sample (0.0424 ± 0.0095 g; $n = 9$; $P > 0.55$; t-test). The color of the furcular fat extracted was variable among birds at all collection times, ranging from beige to light brown.

ULTRASTRUCTURE OF FURCULAR FAT

The ultrastructure of furcular fat varied between November and early April (Fig. 1, Panels A–E). The panels in Figure 1 are arranged from an extensive fat store (1A) to a depleted one (1E). Generally, furcular fat deposits were depleted in birds collected in the morning, while extensive furcular fat deposits characterized chickadees collected in the afternoon. Replete fat cells were unilocular (with one or only a few lipid droplets per cell), had eccentric nuclei, and possessed mitochondrial and capillary densities typical of white adipose tissue. These cells were relatively large, measuring greater than 26.5 μm in diameter. In contrast, depleted cells were multilocular with more centrally placed nuclei, had higher mitochondrial densities, and the capillaries contained more microvilli. Depleted cells were much smaller than replete ones, with diameters between approximately 5.5 and 30 μm. The mitochondria appeared closely associated with the fat droplets in both the replete and depleted adipocytes.

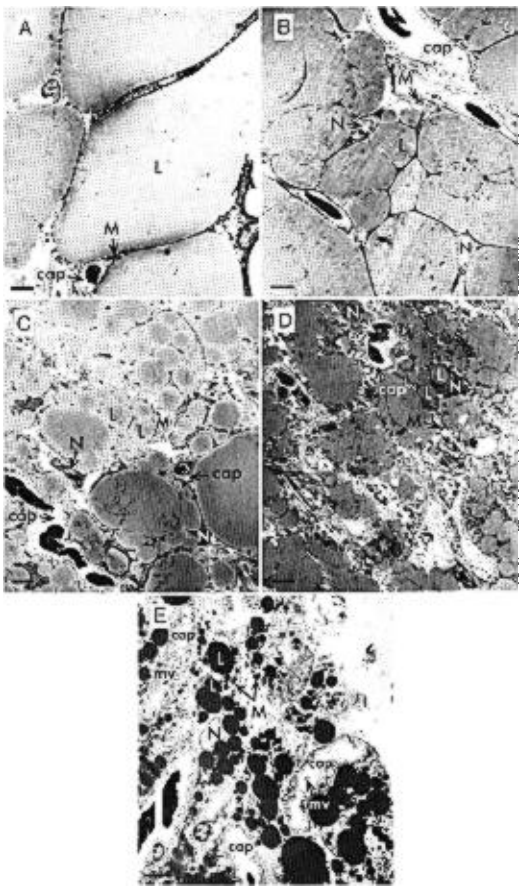

FIGURE 1. Electron micrographs of furcular fat from five different individuals (Panels A–E). The dates and (time of day) of capture of the birds are: 1A—1 November 1984 (p.m.); 1B—3 April 1984 (p.m.); 1C—27 February 1985 (a.m.); 1D—2 March 1985 (a.m.); 1E—26 February 1985 (a.m.). The bars represent 4 μm. The panels are arranged from an extensive fat store (1A) to a depleted one (1E). Replete fat cells are unilocular (with one or only a few lipid droplet(s) (L) per cell), have eccentric nuclei (N), with low mitochondrial (M) and capillary (cap) densities. When compared with replete fat cells, depleted cells are multilocular with more centrally placed nuclei, appear to have higher mitochondrial densities, and have capillaries with more microvilli (mv).

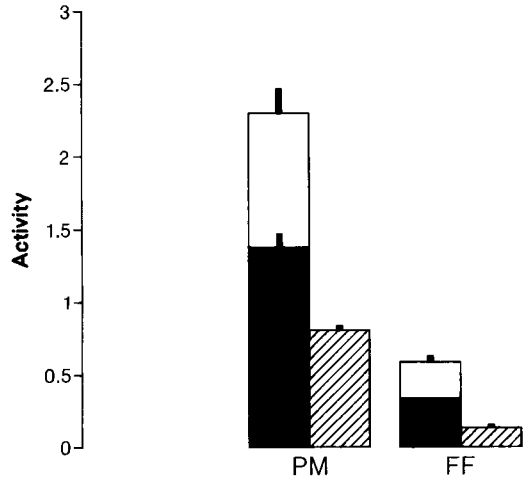

FIGURE 2. Citrate synthase (CS) activity in pectoralis muscle (PM) and furcular fat (FF) of chickadees. CS enzyme activity is presented as μmoles (min·mg protein)$^{-1}$. Black bars—January birds at 25°C; white bars—January birds at 35°C; striped bars—February–March birds at 25°C. Thin vertical bars represent 1 SE.

ACTIVITIES OF CATABOLIC ENZYMES

Comparisons between tissues. CS and HOAD activites (μmoles [min·mg protein]$^{-1}$) in furcular fat were less than 25% and 39% (respectively) of the corresponding activities in pectoralis muscle over all 3 month/assay temperature combinations (January at 25°C, January at 35°C, and February–March at 25°C; Table 1, Figs. 2 and 3). However, no significant differences between tissues exist in the temperature coefficients (Q_{10}) of either enzyme over the range 25–35°C ($P \gg 0.05$). The mean Q_{10} values for CS and HOAD activity in furcular fat were 1.76 and 4.73, respectively, and the corresponding values for pectoralis muscle were 1.70 and 4.59.

Monthly comparisons within tissue. Both pectoralis muscle and furcular fat of chickadees collected in mid-January possessed higher catabolic enzyme activities than the corresponding tissues of birds collected in February–March. Significant differences between the two monthly samples existed in all intratissue comparisons ($P \leq 0.001$), except HOAD activity in pectoralis muscle (Table 1). Furcular fat from January birds possessed a significantly higher concentration of protein (mg protein [g·tissue]$^{-1}$; $P = 0.006$; t-test) from fat collected in February–March, paralleling the higher catabolic capacities in this tissue in January. The concentration of protein in the pectoralis muscle was similar in the two monthly samples.

DISCUSSION

ULTRASTRUCTURE

Recent claims that birds possess brown adipose tissue (Ruffed Grouse and Black-capped Chick-

TABLE 1. Tissue mass, protein concentration, and catabolic enzyme activities for Black-capped Chickadee (*Parus atricapillus*) and white-footed mouse (*Peromyscus leucopus*) tissues. Values for furcular fat (FF) tissue from chickadees captured in January (assayed at 25 and 35°C) and in February–March (assayed at 25°C) are compared with values for BAT from summer- and winter-acclimatized mice (assayed at 25°C; data from Wickler 1979). Statistics for chickadee pectoralis muscle (PM) are also given for comparison. Values are means ± 1 SE, and numbers in parentheses are sample sizes.

Tissue		Mass g	Protein mg (g tissue)$^{-1}$	Citrate synthase μmol (min·mg prot)$^{-1}$	HOAD μmol (min·mg prot)$^{-1}$
Parus atricapillus					
FF—Jan	25°C	0.044 ± 0.010 (9)	78.118 ± 8.758 (9)	0.338 ± 0.028 (9)	0.056 ± 0.008 (9)
	35°C	0.044 ± 0.010 (9)	78.118 ± 8.758 (9)	0.589 ± 0.041 (9)	0.250 ± 0.047 (9)
FF—Feb–Mar	25°C	0.050 ± 0.005 (15)	44.219 ± 6.762 (15)	0.134 ± 0.018 (15)	0.026 ± 0.004 (15)
Peromyscus leucopus					
BAT—Winter	25°C	0.139 ± 0.012 (17)	121.9 ± 13.3 (12)	2.30 ± 0.12 (7)	8.11 ± 0.52 (10)
BAT—Summer	25°C	0.068 ± 0.012 (15)	136.0 ± 12.1 (15)	0.732 ± 0.043 (11)	0.767 ± 0.091 (7)
Parus atricapillus					
PM—Jan	25°C		207.01 ± 4.23 (9)	1.379 ± 0.103 (9)	0.146 ± 0.008 (9)
	35°C		207.01 ± 4.23 (9)	2.299 ± 0.169 (9)	0.658 ± 0.052 (9)
PM—Feb–Mar	25°C		208.06 ± 2.31 (8)	0.808 ± 0.030 (8)	0.116 ± 0.014 (7)

adee, Oliphant 1983; goose, Murphy et al. 1986) have raised the possibility that birds possess mechanisms for regulatory NST similar to those found in mammalian BAT. These claims, however, are based solely on ultrastructural observations of tissues from only one or two individuals per species (one Ruffed Grouse, two chickadees, and one goose). We believe adipose tissues from several conspecific birds must be examined both ultrastructurally and biochemically to establish their similarity with BAT.

Results of the present study demonstrate that the ultrastructure of furcular fat varies considerably in animals collected between November and April in Michigan. For instance, adipocytes in some micrographs are relatively small, multilocular, and appear to possess relatively high mitochondrial densities. These micrographs are similar to those of the periadrenal fat of the Ruffed Grouse and several fat pads of the Black-capped Chickadee provided by Oliphant (1983). Similar multilocular fat cells have been reported in other birds as well (e.g., chicken, Luckenbill and Cohen 1966; pigeon, Lucas and Stettenheim 1972;

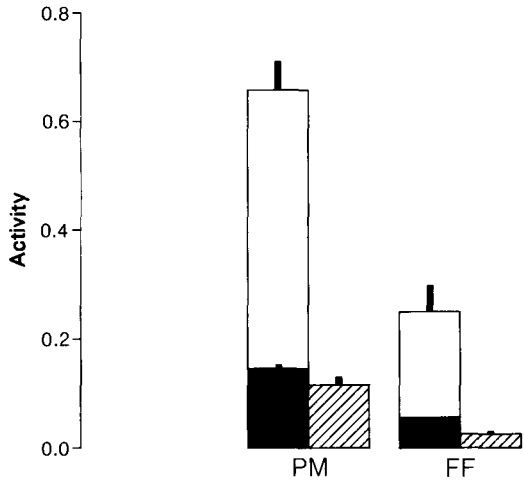

FIGURE 3. β-hydroxyacyl-CoA-dehydrogenase (HOAD) activity in pectoralis muscle (PM) and furcular fat (FF) of chickadees. HOAD enzyme activity is presented as μmoles (min·mg protein)$^{-1}$. Black bars—January birds at 25°C; white bars—January birds at 35°C; striped bars—February–March birds at 25°C. Thin vertical bars represent 1 SE.

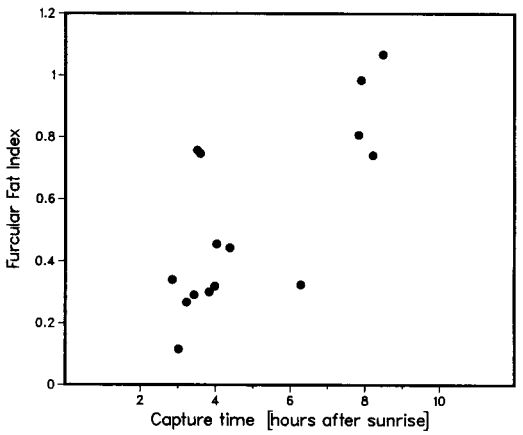

FIGURE 4. Furcular fat index (furcular fat mass as a percent of total body mass) as a function of time animal was collected (hours since sunrise).

ducklings, Barré et al. 1986; goose, Murphy et al. 1986). Adipocytes from other chickadees, however, are larger, unilocular, and exhibit relatively low mitochondrial densities, even though these latter animals were collected within just a few days of the former (see Fig. 1C–E). The variable appearance of furcular fat from chickadees observed over just a few days in this study suggests that the multilocular appearance of this tissue is a function of how much lipid is stored and is not indicative of BAT. Luckenbill and Cohen (1966) and Barré et al. (1986) have also concluded that multilocular fat cells observed in chickens and ducklings (respectively) do not represent BAT.

Although the basis of this intraseasonal variation in adipocyte morphology requires further study, it probably reflects the variable status of energy reserves in the birds, both seasonally and diurnally. The multilocular appearance and seemingly high mitochondrial densities observed in some birds are probably a consequence of lipid depletion. Depleted white fat cells are known to differ from replete cells, changing shape (Napolitano and Gagne 1963) and exhibiting different ultrastructural features (e.g., depleted cells have central or paracentral nuclei; Sheldon 1965). Results of our study provide evidence of a diurnal fluctuation in the fat content of Black-capped Chickadees, as the furcular fat (FF) index (calculated as the percentage of total body mass represented by the mass of furcular fat) is positively correlated to the time of day the birds were captured (calculated as hours since sunrise), especially in January–February (Fig. 4). Similar diurnal increases in fat stores have been observed in populations of Black-capped Chickadees from northern Michigan (T. Van't Hof, pers. comm.) and New York (Chaplin 1974) as well as in other small passerine birds, e.g., the House Finch (*Carpodacus mexicanus,* Dawson et al. 1983) and American Goldfinch (*Carduelis tristis,* R. L. Marsh, pers. comm.). The marked interindividual variation in the FF index as a function of time of day observed in chickadees collected in the cold months of January–February (0.12 to 1.07%, an 8.9-fold difference; Fig. 4), together with the documented ability of avian adipose tissue to rapidly store (through esterification of fatty acids) and subsequently mobilize lipid (Leveille et al. 1975) suggest that fat is an important substrate during cold exposure. The energetic demands associated with thermoregulation would be especially severe during the night. Therefore, we believe the multilocular appearance of the depleted fat cell and the abundant microvilli in the adjacent capillaries of birds collected in the morning (Fig. 1) reflect high rates of lipid mobilization during and immediately after the nocturnal fast. Barré et al. (1986) reported similar ultrastructural changes in the capillaries of the fat depots of both cold-acclimated and fasted ducklings relative to controls.

Concomitant with the increase in the FF index is a corresponding decrease in protein concentration ($r^2 = 0.50$; $P < 0.001$; $n = 24$). The pronounced inverse relationship between protein concentration and fat mass suggests lipid is added without an attendant synthesis of protein. In contrast, protein concentrations in interscapular BAT from summer- and winter-acclimatized *Peromyscus* are similar, although the total mass of the fat changes considerably between seasons (Wickler 1979). The furcular fat depot in Black-capped Chickadees thus appears to hypertrophy primarily with fat as the bird feeds throughout the day, without the concomitant increase in tissue protein that accompanies increases in BAT mass in *Peromyscus.* Similar lipid accumulations in avian adipose tissue without attendant increases in the nonlipid components of the cell occur in Black-capped Chickadees in New York during winter (Chaplin 1974) and during migration (Odum et al. 1964, Odum 1965, Johnston 1973).

CATABOLIC CAPACITY

The use of CS and HOAD as indices of aerobic and β-oxidative capacities is based on their either catalyzing nonequilibrium reactions or being closely associated with the activity of a nonequilibrium enzyme in the same pathway (Newsholme and Start 1973). The in vitro activities of CS and HOAD correlate with the capacity of an individual tissue to use substrate (Marsh 1981), and therefore serve as useful biochemical markers of tissue and seasonal differences in the maximum flux (i.e., capacity) through the TCA cycle and β-oxidative pathways, respectively.

TCA cycle and β-oxidative capacities of chickadee furcular fat are below 40% (January) or below 23% (February to March) of the corresponding values in pectoralis muscle. These differences persist even at assay temperatures (35°C) within the range of body temperatures sometimes evident in Black-capped Chickadees during nocturnal hypothermia (Chaplin 1974, 1976).

More pronounced differences exist between the catabolic capacities of chickadee furcular fat and mammalian BAT (see Wickler 1979). TCA cycle and β-oxidative capacities (assayed at 25°C) in furcular fat from winter-acclimatized chickadees are only 14.7% and 0.7% of the corresponding values in BAT from winter-acclimatized *Peromyscus leucopus* (Table 1). These differences in catabolic capacity persist even when comparisons are made with summer-acclimatized mice, despite the fact that these mice were not challenged by cold; TCA cycle and β-oxidative capacities of furcular fat are only 46.2% and 7.3% of the comparable values in BAT from summer-acclimatized mice. The relatively low capacities for the oxidation of fatty acids present in furcular fat from the chickadee do not support a role for this tissue as a site of intense thermogenesis (cf. Wickler 1979, see Himms-Hagen 1985 for a review). Furthermore, the strong thermal dependence of HOAD activity over the range 25–35°C suggests that it is unlikely that thermogenesis in furcular fat contributes substantially to the rewarming of chickadees arousing from nocturnal hypothermia.

The existence of monthly differences in the catabolic potential of the furcular fat tissue of winter-acclimatized Black-capped Chickadees is intriguing. Higher activities of the catabolic enzymes in January birds (Table 1) could reflect higher flux through both the TCA cycle and β-oxidative pathways during this month than in February–March. Perhaps this helps support higher rates of lipid mobilization in this tissue during January (Barré et al. 1986). Increased production of ATP would be needed under such conditions to provide substrate for initiating lipolysis. Equally interesting is the observed increase in TCA cycle capacity of the pectoralis muscle without a concomitant increase in β-oxidative capacity. This seasonal change in catabolic profile contrasts with the situation in American Goldfinches (*Carduelis tristis*), where HOAD activity is significantly higher in winter than in spring, but CS activity remains constant (Yacoe and Dawson 1983). The significance of these interspecific differences requires further study.

CONCLUSIONS

The relatively low CS and HOAD activities in furcular fat from Black-capped Chickadees (especially relative to those in mammalian BAT) indicate that this tissue has a low catabolic potential. Furthermore, catabolic capacities remain low, even at assay temperatures near the body temperature of these birds, despite large changes in the mass of furcular fat. This demonstrates that lipid is stored without an attendant increase in catabolic potential. These observations, together with the wide morphological fluctuations occurring in furcular fat within the winter season (e.g., in the amount of fat stored and the associated changes in vascularity and degree of multilocularity), suggest that this tissue serves primarily as a fat depot rather than as a site of intense thermogenesis, which would require rapid catabolism of endogenous lipid. Therefore, we conclude that this tissue differs functionally from typical mammalian BAT. We regard an alternative hypothesis concerning its function as more plausible: the ultrastructure and biochemistry of furcular adipose tissue in chickadees reflects a primary role of avian adipose tissue in mobilizing substrate (e.g., see Oliphant 1983: 354; Barré et al. 1986). Finally, it is important to note that even though the observations summarized here appear to refute claims for a role of BAT in avian thermogenesis, they do not exclude the possibility of regulatory NST in birds. However, the site and mechanisms for any such NST remain to be identified.

ACKNOWLEDGMENTS

We thank Thomas J. Van't Hof for helping collect the birds used in this study. J. C. George provided helpful criticism early in the investigation, and two anonymous reviewers provided helpful comments on the manuscript. This study was supported in part by NSF Grant BSR 84-07592.

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